Role of epoxyeicosatrienoic acids in renal functional response to inhibition of NO production in the rat

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Ogungbade, G. O., L. A. Akinsanmi, H. Jiang, and A. O. Oyekan. Role of epoxyeicosatrienoic acids in renal functional response to inhibition of NO production in the rat. Am J Physiol Renal Physiol 285: F955–F964, 2003. First published July 15, 2003; 10.1152/ajprenal.00092.2003.—Nitric oxide (NO) inhibits hemoproteins, including cytochrome (CYP) 2C, the gene responsible for the production of epoxyeicosatrienoic acids (EETs). EETs and NO are produced in the kidney, and both regulate renal vascular tone and Na+ transport. However, the role of EETs in NO-mediated renal function is not known. This study tested the hypothesis that NO tonically regulates the renal production of EETs, thereby impacting renal vasomotor tone and electrolyte balance. LPS (10 mg/kg iv) inhibited microsomal conversion of 14C-labeled arachidonic acid to EETs and reduced mean arterial blood pressure (MABP; Δ = 63 ± 5 mmHg). Nitro-L-arginine methyl ester (L-NAME, 10 mg/kg), an inhibitor of NO synthase, increased MABP (Δ = 26 ± 6 mmHg), reduced cortical (CBF) and medullary (MBF) blood flow (Δ = −0.86 ± 0.15 and −0.34 ± 0.09 V, respectively) and glomerular filtration rate (GFR; from 0.82 ± 0.16 to 0.32 ± 0.10 ml/g kidney − min −1), and increased Na+ excretion (UNaV, from 0.16 ± 0.04 to 0.30 ± 0.06 μmol·g kidney −1·min −1). 2-(2-Propynoloxy)-benzenenehexaonoic acid (PPOH), a suicide substrate inhibitor of EET production, did not affect the L-NAME-induced increase in MABP but attenuated the effects of L-NAME on CBF (31 ± 7%, P < 0.05%), GFR (44 ± 6%, P < 0.05), and UNaV (78 ± 7%, P < 0.05). Miconazole (1.3 mg·kg −1·h −1), a heme inhibitor of epoxygenase enzymes, produced effects similar to those of PPOH. Renal intrarterial infusion of 5.6-, 8.9-, 11.12-, and 14.15-EET (1–10 ng/min) elicited dose-dependent reductions in CBF and GFR accompanied by regioisomeric changes in MBF, UNaV, and urine flow rate. In addition, 11,12-EET dose dependently restored the PPOH blunting the effects of L-NAME on CBF, MBF, and GFR. We conclude that NO tonically regulates epoxygenase activity and that EETs are renal vasocostricators in vivo and contribute, at least in part, to the renal functional responses following inhibition of NO production. cytome P-450; kidney

Nitric oxide (NO) is an important endogenous regulator of renal vascular tone and ion transport and, therefore, has a number of effects that have an impact on the regulation of renal function and blood pressure (3). It has been implicated in renal autoregulation (7, 8), tubuloglomerular feedback (39, 44), and pressure natriuresis (13, 17). Its physiological actions are attributable to the oxidation of heme and nonheme iron and iron-sulfur complexes in the active sites of key metabolic enzymes, especially heme-containing enzymes, such as NO synthase (NOS) and cytochrome P-450 (CYP) enzymes, including CYP1A1, CYP2B1, and CYP3A (20), CYP4A (1, 28), and CYP2C (6, 21, 36, 37) isoforms, which produce epoxyeicosatrienoic acids (EETs, epoxides). NOS was the first example of a mammalian soluble isomerase of CYP reductase containing a reductase and a heme domain on the polypeptide (43). Both enzymes are flavoproteins with an amino acid sequence that is 36% identical and 58% homologous (5), making CYP reductase the only known mammalian enzyme with close homology to NOS (5, 43). This, therefore, raises the possibility for interactions among NO and CYP products.

Epoxides are CYP epoxidation products of arachidonic acid (AA) that are endogenous to the kidney and occur in plasma and urine of rats and humans (14, 32). On the basis of their biological properties, which include changes in vasoactivity, modulation of ion transport, and cell growth (14, 32), a major role was proposed for epoxides in cardiovascular and renal function, including a role in pressure-natriuresis (27) and in salt-sensitive (23) and angiotensin II-induced hypertension (15). Similar to NO, epoxygenase metabolites of AA are produced in the kidney and affect vascular tone and electrolyte balance (14, 32). The coproduction and actions on the kidney of EETs and NO raise the specter for interactions between both autocrine mediators. Thus these studies evaluated the functional implications of NO-EET interactions in the kidney by testing the hypothesis that NO interaction with epoxygenase arachidonate metabolites is a key component in the homeostatic control of renal function and regulation of blood pressure. Epoxides or their metabolites elicit renal vasoconstriction (9, 38) and promote natriuresis (38) and/or mediate hormone-induced natriuresis (33). These studies, therefore, evaluated whether the responses following inhibition of NO production

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may be associated with increased production of epoxides. We reasoned that if NO tonically inhibits epoxygenase enzyme activity, inhibition of NOS would be expected to increase formation of epoxides that should contribute to the renal hemodynamic and excretory effects after withdrawal of NO, and suppression of epoxygenase metabolism should inhibit these renal effects.

To this end, we evaluated the effects of NO on NADPH/epoxygenase-dependent renal microsomal metabolism of AA as well as the effects of epoxygenase inhibition on changes in renal functional response to withdrawal of endogenous NO. Our data demonstrated inhibition on changes in renal functional response to NADPH/epoxygenase-dependent renal microsomal metabolism should inhibit these renal effects after withdrawal of NO, and suppression of epoxygenase enzyme activity, inhibition of NOS would be evidenced in rats 1 and 2 h after treatment with miconazole and PPOH, respectively, or their vehicles.

Enzyme activity was calculated from percent conversion of [14C]AA in individual microsomal preparations.

In all cases, metabolites were extracted with acidified ethyl acetate (pH 3–4) and subjected to reverse-phase high-performance liquid chromatography (HPLC). The elution profile of the radioactive metabolites of AA was detected and counted using an online radioactive detector (Radiomatic Instruments, Tampa, FL). The identity of each metabolite was confirmed by its comigration with authentic standards on the HPLC. Total epoxygenase metabolites were quantified by pooling products emerging with retention times of 13.5–14.7 min (epoxides) and 6.7–7.3 min (diols).

**Urinary Excretion of Epoxides**

Rats were placed in metabolic cages, and 24-h urine samples were collected after 10 days from rats (n = 4–5/group) treated with vehicle (15% DMSO, control), PPOH (5 mg/day) bolus iv), l-NNA (0.5 g/l in drinking water for 10 days), l-NNA + PPOH, or SNAP (50 nmol·kg⁻¹·min⁻¹ by osmotic minipump). The dose of SNAP was based on preliminary studies in which we demonstrated that, in anesthetized rats, 6 h of infusion with SNAP at 30 and 100 nmol·kg⁻¹·min⁻¹ produced an 11 ± 4 and 39 ± 7% reduction in CBF, respectively. The vehicle or PPOH was administered under light halothane anesthesia. Concentration of epoxides was determined following a modification of the negative chemical ionization gas chromatography-mass spectrometry (GC-MS) that we previously published (28). Briefly, epoxides in urine samples (10 ml) were initially purified by thin-layer chromatography using the A9 solvent system followed by HPLC fractionation and quantitation by GC-MS. Because all four isomers of EETs, 5,6-, 8,9-, 11,12-, and 14,15-EET, show up as urinary DHETs, respectively. The derivatization procedure was the same as that previously reported for 20-HETE (28). Authentic standards were used to identify the specific EETs. The
5.6-, 8.9-, and 11.12-diHETEs showed up with the same retention time on GC-MS, whereas the 14.15-diHETE appeared as a separate peak. The concentrations of EETs were quantitated together by calculating their concentrations from the peak area ratios of m/z 481 to m/z 489 according to the standard curve of DHET vs. DHET-d₄s.

**Physiological Measurements**

Clearance studies were performed in rats that were anesthetized with an injection of thiobutabarbital (Inactin, 100 mg/kg ip; Research Biochemicals International, Natick, MA) and placed on a heated platform to maintain body temperature at 37°C. The urinary bladder was exposed by an abdominal incision, and a cannula (PE-205) was placed to drain urine. Dead space was minimized by a ligature that functionally excluded a great part of the bladder. A tracheostomy (PE-250) was performed for spontaneous ventilation, and cannulas (PE-50) were placed in the right carotid artery to monitor blood pressure and the right femoral artery to collect blood samples. A tail vein was also cannulated with a 23-gauge needle (Abbott Hospitals) for infusion or administration of drugs. In some experiments, drugs were administered by renal intra-arterial infusion using a 30-gauge needle that was placed in the left renal artery through a left laparotomy. A constant infusion of saline (2 ml/h) was administered intravenously throughout the experiment to maintain a euvolemic condition. Inulin (10 mg/ml) was included in the solution for measurement of glomerular filtration rate (GFR). Mean arterial blood pressure (MAPB) was measured with a pressure transducer (model P231D, Statham, Oxnard, CA) to a signal manifold (Transbridge, model TBM-4, World Precision Instrument, Sarasota, FL) and recorded on a data acquisition system (model DI720, Dataq Instruments, Akron, OH). In some experiments, cortical and medullary blood flows (CBF and MBF, respectively) were measured simultaneously by laser-Doppler (LD) flowmeter (system 5000, version 1.20, Periflux, Stockholm, Sweden) via a surface probe (model PF 407) to measure CBF or an optical fiber LD probe (model PF 402) fixed to a micromanipulator and placed in the medulla (5 mm below the kidney surface) to measure MBF. CBF and MBF were obtained as perfusion units (PU) and expressed as volts (100 PU corresponding to 1 V). The flowmeter was calibrated using a colloidal suspension of latex particles (Permed Motility Standard), which, at room temperature, gives a signal of 250 PU (2.5 V, ±5%). At the end of the experiment, the renal artery was completely occluded to obtain a zero-flow reading in the LD flowmeter, and this value, 30 PU (0.03 V), for the cortex or 14 PU (0.014 V) for the medulla was subtracted from the signal recorded during the experiment.

The rats were allowed to equilibrate for ≥45 min or until urine flow was steady. After this postsurgical equilibration period, a 30-min control clearance period was obtained. This was followed by one or more experimental clearance periods (30 min each), during which the effects of L-NAME or epoxides (5.6-, 8.9-, 11.12-, or 14.15-EET) on MABP, CBF, MBF, and GFR were evaluated. L-NAME (10 mg/kg) was administered by slow intravenous injection. This dose was selected on the basis of our previous study (29) indicating that the 10 mg/kg dose evoked the maximal increase in renal vascular resistance. In some experiments, responses to a nonpressor dose of L-NAME (1 mg/kg iv) were evaluated. A time control group (n = 4) was treated with an identical injection of normal saline (1 ml/kg) not containing L-NAME (control). In experiments in which the renal effects of epoxides were determined, 5.6-, 8.9-, 11.12-, or 14.15-EET (1, 3, and 10 ng/min) or their vehicle (10% methanol, final concentration) was administered by renal intra-arterial infusion at a flow rate of 2.5 μl/min for 30 min. In all cases, changes in MABP, CBF, and MBF were continuously monitored. Urine samples were also collected. Arterial blood (400 μl) was collected into 3.5% sodium citrate during the middle of a 30-min urine collection period for measurement of GFR by inulin clearance or 1, 3, and 10 ng/min of L-NAME or L-NNA. These times and doses are based on published data indicating that they are the pretreatment periods for optimal inhibition of epoxygenase (4, 27). Control rats (n = 5) received 15 or 25% DMSO (1 ml/kg iv) for 1 or 2–3 h. Data obtained from rats treated with 25% DMSO, the vehicle for miconazole, were not different from data obtained from rats treated with 15% DMSO, the vehicle for PPOH; hence, data from both groups of rats were pooled to represent control data for the miconazole and PPOH treatment groups.

**Experimental Groups**

Experiments were performed on seven groups of rats as follows.

- **Group I.** For the time control for L-NAME or L-NNA (n = 4), baseline measurements were recorded (0–30 min) and repeated during an experimental period (30–60 min) after the administration of 0.9% NaCl (normal saline).
- **Group II.** Renal responses were determined in rats that received bolus doses of L-NAME (1 and 10 mg/kg iv) or L-NNA (10 mg/kg iv) given slowly over 1 min after the baseline period (0–30 min).
- **Groups III and IV.** The epoxygenase enzyme was inhibited by miconazole or PPOH. Miconazole or PPOH was administered 1 or 2–3 h, respectively, before injection of L-NAME or L-NNA. These times and doses are based on published data indicating that they are the pretreatment periods for optimal inhibition of epoxygenase (4, 27). Control rats (n = 5) received 15 or 25% DMSO (1 ml/kg iv) for 1 or 2–3 h. Data obtained from rats treated with 25% DMSO, the vehicle for miconazole, were not different from data obtained from rats treated with 15% DMSO, the vehicle for PPOH; hence, data from both groups of rats were pooled to represent control data for the miconazole and PPOH treatment groups.
- **Group V.** Doses of 5.6-, 8.9-, 11.12-, and 14.15-EET were given renal intra-arterially in a cumulative fashion. The administration of each of these agents was preceded by the administration of 10% methanol for 30 min (control).
- **Group VI.** For the time control for the epoxide-treated group (n = 4), 10% methanol (vehicle) was continuously infused at 2.5 μl/min, and GFR, CBF, and MBF were determined at 3 h. The responses were compared with those obtained at the beginning of the first 30 min of the infusion.
- **Group VII.** PPOH and L-NAME were administered 5 min after commencement of renal intra-arterial infusion of vehicle or 1, 3, and 10 ng/mg of 11.12-EET.

Independent effects of PPOH or miconazole were evaluated by comparing renal function during the baseline period between vehicle- and inhibitor-treated groups. The effects of PPOH or miconazole on L-NAME- or L-NNA-induced changes in renal function were evaluated by comparing renal effects of L-NAME or L-NNA in the presence of an inhibitor or its vehicle.

**Statistical Analysis**

Renal microsomal epoxygenase activities are expressed as percentages of the control values. Hemodynamic responses are recorded as changes (Δ) relative to baseline. Values of GFR are presented as absolute values. Values are mean ± SE. Areas under the curve for changes in hemodynamic values vs. time were determined using regression analysis. Hemodynamic and microsomal data were analyzed by analysis of variance followed by a Newman-Keuls test or Stu-
dent’s *t*-test for paired or unpaired data (GraphPad version 3.0) as appropriate to determine significance between control and treated groups. In all cases, *P* < 0.05 was regarded as significant.

**RESULTS**

**Effect of LPS, l-NNA, Miconazole, and PPOH on Renal Microsomal CYP- AA Metabolism**

In whole renal microsomes from untreated rats incubated with 3 μM [14C]AA (0.2–0.4 μCi), 10 μM indomethacin, and 1 mM NADPH, control epoxygenase activity was 1.48 ± 0.4 pmol·mg protein⁻¹·30 min⁻¹. In rats treated with LPS, renal microsomal epoxygenase activity decreased by 81 ± 8% (*P* < 0.05; Fig. 1A). In addition, LPS treatment reduced MABP by −63 ± 5 mmHg from a pretreatment value of 109 ± 5 mmHg vs. a reduction of −5 ± 3 mmHg in vehicle-treated (*n* = 6) rats (*P* < 0.05). Compared with microsomes harvested from vehicle (DMSO)-treated rats, PPOH or miconazole, epoxygenase inhibitors, blunted conversion of [14C]AA to epoxygenase metabolites by 64 ± 5% (*P* < 0.05, *n* = 5) and 45 ± 6% (*P* < 0.05, *n* = 7), respectively. In whole kidney microsomes prepared from rats (*n* = 5) treated with l-NNA (0.5 g/l for 10 days), total epoxygenase activity increased by 20 ± 4% (*P* < 0.05) compared with microsomes from control rats. l-NNA also increased SBP from 123 ± 4 mmHg on day 1 to 153 ± 7 mmHg (*P* < 0.05) on day 10.

**Effects of PPOH, NO Donor, and NOS Inhibitor on Renal Production of Epoxides**

Figure 1B illustrates the excretion of epoxides in urine samples collected from rats treated with vehicle (control), PPOH, SNAP, l-NNA, or l-NNA + PPOH. Excretion of epoxides in vehicle-treated rats was 252 ± 60 ng/day. PPOH or SNAP blunted epoxide excretion by 42 ± 8% (*P* < 0.05) and 54 ± 6% (*P* < 0.05), respectively. l-NNA slightly increased the excretion (16 ± 7%), but this did not reach significant levels. However, PPOH blunted the excretion of epoxides in l-NNA-treated rats to levels that were not different from those obtained in rats treated with PPOH alone.

**Renal Responses to Administration of Inhibitors of NO Production**

Basal MABP, CBF, MBF, and GFR in the rats (*n* = 10) used in these experiments were 109 ± 4 mmHg, 4.32 ± 0.32 V, 1.61 ± 0.21 V, and 0.85 ± 0.14 ml·min⁻¹·g kidney⁻¹, respectively. Injection of l-NNAME (10 mg/kg) produced a rapid and pronounced increase in MABP (*Δ* = 26 ± 6 mmHg) and reductions in CBF (*Δ* = −0.86 ± 0.15 V) and MBF (*Δ* = −0.34 ± 0.09 V; data not shown) that were sustained for ≥30 min (Fig. 2A). l-NNAME also reduced GFR by 58 ± 5% (*P* < 0.05) from 0.82 ± 0.16 to 0.32 ± 0.10 ml·min⁻¹·g kidney⁻¹ (Fig. 2C). Similarly, l-NNAME (10 mg/kg) elicited sustained increases in MABP (*Δ* = 34 ± 6 mmHg) that were accompanied by reductions in CBF (*Δ* = −0.93 ± 0.21 V) and MBF (*Δ* = −0.40 ± 0.07 V). In time controls (control, *n* = 4), there were no differences in MABP, CBF, MBF, and GFR when these parameters were evaluated 60 min after the administration of normal saline: 112 ± 6 mmHg, 4.22 ± 0.18 V, 1.44 ± 0.09 V, and 0.77 ± 0.12 ml·min⁻¹·g kidney⁻¹, respectively. In rats treated with 15% DMSO (*n* = 6), the vehicle for PPOH, or 25% DMSO (*n* = 2), the vehicle for miconazole, pooled values of MABP, CBF, MBF, and GFR were 111 ± 6 mmHg, 4.28 ± 0.27 V, 1.47 ± 0.15 V, and 0.74 ± 0.11 ml·min⁻¹·g kidney⁻¹, respectively, which were not significantly different from values obtained from rats (*n* = 4) that received normal saline.

**Effect of Inhibition of Epoxygenase Activity During Acute l-NNAME Administration**

Basal values of MABP, CBF, and GFR in PPOH-treated rats were 108 ± 5 mmHg, 4.11 ± 0.21 V, and 0.92 ± 0.14 ml·min⁻¹·g kidney⁻¹, respectively. MABP and CBF values were not different from values in rats treated with the vehicle. However, GFR increased (from 0.74 ± 0.11 to 0.92 ± 0.14 ml·min⁻¹·g kidney⁻¹).
and basal MBF was 25 ± 5% (P < 0.05) greater in PPOH- than in vehicle-treated rats (1.47 ± 0.15 vs. 1.85 ± 0.19 V; data not shown). For miconazole, basal values of MABP, CBF, or GFR were not changed compared with values from vehicle-treated rats, but basal MBF was greater (1.43 ± 0.12 vs. 1.68 ± 0.14 V) in miconazole- than in vehicle-treated rats (data not shown). Figure 2 shows that PPOH did not affect the L-NAME-induced increase in MABP but attenuated the reductions in CBF (31 ± 7%, P < 0.05). This effect was accompanied by attenuation of the L-NAME-induced reduction in GFR (44 ± 6%, P < 0.05), such that the L-NAME-induced reduction in GFR in PPOH-treated rats was 32 ± 5% compared with 58 ± 5% in vehicle-treated rats (Fig. 2C). Miconazole also did not affect the L-NAME-induced increase in MABP (140 ± 6 vs. 135 ± 8 mmHg) but attenuated the L-NAME-induced reductions in CBF from 4.21 ± 0.42 V to a nadir of 3.34 ± 0.28 V (P < 0.05) and in MFB from 1.54 ± 0.14 to 1.35 ± 0.11 V (P < 0.05; data not shown). Unlike these effects, L-NAME (1 mg/kg) did not increase MABP (Δ = 2 ± 2 mmHg) but reduced CBF (Δ = −0.21 ± 0.06 V) and MFB (Δ = −0.09 ± 0.04 V). In addition, this dose of L-NAME increased UV and UNaV by 14 ± 4% and 18 ± 7%, respectively, from basal values of 0.24 ± 0.06 µl·min⁻¹·g kidney⁻¹ and 0.17 ± 0.07 µmol·min⁻¹·g kidney⁻¹, respectively (P < 0.05). PPOH attenuated the effects of L-NAME on CBF, MBF, UV, and UNaV by 18 ± 5% (P < 0.05), 22 ± 6% (P < 0.05), 34 ± 7% (P < 0.05), and 39 ± 6% (P < 0.05), respectively (data not shown).

Renal Effects of Epoxides

Figure 3 illustrates the renal effects after renal intra-arterial administration of the four regioisomeric epoxides on GFR, UV, and UNaV. Control values of these parameters in rats that received renal intra-arterial infusion of 10% methanol (vehicle) were 0.55 ± 0.06 ml·min⁻¹·g kidney⁻¹, 0.28 ± 0.05 µl·min⁻¹·g kidney⁻¹, and 0.23 ± 0.04 µmol·min⁻¹·g kidney⁻¹, respectively. There were no effects on MABP by any of the EETs at any of the doses. However, 5,6-, 8,9-, 11,12-, and 14,15-EET at 1, 3, and 10 ng/min reduced GFR in a dose-dependent manner. The greatest effect on GFR was observed with 11,12-EET, which, at 3 ng/min, elicited a marked reduction (73 ± 5%, P < 0.05) in GFR. Of the EETs, 5,6-EET produced the least effect on GFR, inasmuch as it produced a modest 26 ± 6% reduction (P < 0.05), even at the highest concentration, 10 ng/min, at which the other EETs produced >50% reduction in GFR. The effects of the EETs on UNaV and UV are distinct, inasmuch as 8,9-EET produced antidiuretic/antinatriuretic effects, reducing UNaV and UV by 58 ± 7% (P < 0.05) and 63 ± 8% (P < 0.05), respectively, at 10 ng/min, whereas the other regioisomers of EET, namely, 5,6-, 11,12-, and 14,15-EET, produced dose-dependent increases in UNaV and UV. 11,12-EET is the most potent of these, producing a fourfold increase in UNaV and a twofold increase in UV at 10 ng/min.

Renal Hemodynamic Effects of Epoxides

The effects of 5,6-, 8,9-, 11,12-, and 14,15-EET on regional hemodynamics are presented in Fig. 4. Basal CBF and MBF in the rats (n = 4–6) used in these experiments were 4.28 ± 0.29 and 1.31 ± 0.16 V. All four regioisomers of EET produced dose-dependent reductions in CBF; 8,9-EET was the least potent, producing a modest 12 ± 3% peak reduction in CBF at the highest dose employed, whereas 14,15-EET was the most potent, producing a 24 ± 5% peak reduction in CBF.
at 10 ng/min. Unlike the qualitatively similar effects produced by these epoxides on CBF, the effects on MBF are distinct, inasmuch as 8,9-EET produced medullary perfusion at higher doses, increasing MBF to peak values of 0.39 ± 0.11 and 0.62 ± 0.22 V at 3 and 10 ng/min, respectively. On the other hand, the low dose of 8,9-EET (1 ng/min) and the other EETs produced dose-dependent reductions in MBF. The effects of 5,6-, 11,12-, and 14,15-EET on MBF are about equal in potency, producing a 18–22% reduction in MBF at the highest dose.

Renal Effects of 11,12-EET in the Presence of L-NAME

Inasmuch as 11,12-EET produced potent effects on CBF and GFR, its ability to restore L-NAME-induced renal responses in rats treated with PPOH were evaluated. Figure 5 illustrates that 11,12-EET produced dose-dependent reductions in CBF, MBF (data not shown), and GFR in rats that were treated with PPOH and L-NAME. A full restoration of the effect of L-NAME on these parameters was obtained at 3 ng/min.

DISCUSSION

NO has been implicated in the paracrine control of renal hemodynamics (3, 25) and the regulation of salt and water excretion (8, 13). The diverse actions of NO and its effects on the multiple cell types in the kidney have led to the assignment of a pivotal role for NO in the complex integrated functions of the kidney. There is abundant evidence to support the concept that tonically generated NO plays a major modulatory role in the regulation of vasomotor tone and fluid and electrolyte balance (3, 7). Thus systemic NOS inhibition with L-arginine analogs resulted in vasoconstriction and produced dose-dependent increases in arterial pressure, indicating that constitutive release of NO maintains the vasculature in a partially dilated state (41). The vasoconstriction observed in response to L-arginine analogs persisted for the duration of NOS inhibition, suggesting that NO sets the level of tone at which the other control systems operate. Thus withdrawal of an active NO vasodilator stimulus produces vasoconstriction directly or amplifies underlying vasoconstrictor systems, for example, the renin-angiotensin (10) and sympathetic nervous systems (40), and endothelins (2, 31). Studies from our laboratory and others have also demonstrated that NO inhibition amplifies the CYP ω-hydroxylase system (1, 29), suggesting that these vasoconstrictor/prohypertensive systems are kept in abeyance by the tonic modulatory activity of NO.

Our previous study demonstrated that 20-HETE is the CYP-AA product that best fits the biological profile of the mediator of renal functional responses to NOS inhibition (29). However, like 20-HETE, epoxides produce renal vasoconstriction (9) while promoting salt and water excretion (38) and, therefore, could also contribute to renal responses to NOS inhibition. EETs are produced in the preglomerular arterioles, glomerulus, proximal tubule, and pars recta (32) and are the primary CYP metabolites of AA produced in the collecting duct (12). Inasmuch as NO is produced at the same sites (24), the potential for interactions is great. Hence, in this study we have tested the hypothesis that NO exerts a braking action on renal CYP-dependent epoxygenase metabolism and thereby affects the regulation by epoxides of renal vasomotor tone and fluid and electrolyte balance. As a first step to testing this hypothesis, we established NO inhibition of epoxygenase activity by demonstrating that treatment of rats with LPS, a potent inducer of NO, markedly diminished epoxygenase activity. In addition, SNAP, an NO donor, reduced renal excretion of epoxides. These observations are in agreement with the findings that NO blocks the synthesis of EETs (1, 19, 30) and are con-
consistent with the demonstration that NO inhibits the expression of enzymes of the 2C and 3A families (36, 37). Additional evidence for NO inhibition of microsomal epoxygenase activity was provided by the demonstration in this study that long-term inhibition of NOS after treatment of rats with L-NNA was accompanied by increased renal microsomal epoxygenase activity. In addition, L-NNA tended to increase renal excretion of epoxides. However, this was not significant, a finding that may reflect their autocrine nature, in that they are very lipophilic and, therefore, are found in higher concentrations within tissues than in plasma, urine, or interstitial fluid. Nonetheless, the significant increase by L-NNA in microsomal epoxygenase activity is consistent with our previous study in which L-NAME treatment increased renal medullary epoxygenase activity (30). Considering that LPS inhibition of epoxygenase activity was accompanied by hypotension, we deduced that LPS-induced hypotension was due to inhibition of one or more vasoconstrictor/prohypertensive epoxygenase product(s). This conclusion is consistent with the demonstration that inhibition of a vasoconstrictor CYP product, namely, 20-HETE, contributes to the vasodilator and renal response to NO in vivo (1). However, inasmuch as LPS or NO inhibited epoxygenase activity in these and other studies (6, 36), it appears that inhibition of one or more vasoconstrictor epoxygenase metabolite(s) contributes to the hypotensive effect of LPS. This conclusion is supported by the fact that epoxides or its metabolites produce vasoconstriction in the blood-perfused kidney (38). In many tissues, EETs can be further metabolized by CYP, lipoxygenase, and cyclooxygenase (COX) enzymes. For example, 11,12- and 14,15-EET are metabolized by.

Fig. 4. Changes in cortical (CBF) and medullary blood flow (MBF) after renal intra-arterial infusion of 5,6- (A), 8,9- (B), 11,12- (C), or 14,15-epoxyeicosatrienoic acid (EET; D) at 1, 3, and 10 ng/min or vehicle infusion (0 ng/min) for 30 min each. *P < 0.05 vs. 0 ng/min.
were pretreated 3 h before administration of 11,12-EET. *P vs. 0 ng/min; #P vs. L-NAME (10 mg/kg) in rats (9).

Renal intra-arterial infusion of 11,12-EET was initiated 5 min before administration of these thromboxane receptor antagonists (38). Consistent with these findings, we observed that inhibition of epoxygenase activity diminished the hypotensive effect of 5,6-EET by platelet-derived COX to vasoconstrictor endoperoxides (9). These observations support the demonstration that EETs elicited renal vasoconstriction in vivo that was blocked by COX inhibitors and thromboxane receptor antagonists (38). Consistent with these findings, we observed that inhibition of epoxygenase activity diminished the hypotensive effect of LPS in the anesthetized rat (unpublished observation). As additional evidence for NO inhibition of epoxygenase activity, we proposed that, in the euvoletic rat, L-NAME administration and the consequent withdrawal of the tonic inhibition by NO of epoxygenase activity allowed a full expression of the renal functional effects of epoxides. We obtained evidence that these products must arise from epoxygenase activity, inasmuch as the renal effects were diminished by PPOH, a selective epoxygenase substrate inhibitor, or miconazole, an inhibitor of the heme moiety of CYP enzymes (42). Thus, in the present study, L-NAME-induced decreases in CBF, MBF, and GFR, as well as natriuresis, were attenuated by PPOH. The ability of PPOH to diminish the effects of L-NAME supports a role for epoxides in the effects. The natriuresis produced in response to L-NAME is similar to our previous finding (29) and those of Johnson and Freeman (17) in conscious rats and Ziyyat et al. (45) in the rat isolated kidney. It appears that the natriuresis following NO inhibition does not derive only from its ability to increase blood pressure, because a nonpressor dose of L-NAME also increased natriuresis. The inhibition by PPOH of the effect observed on salt and water excretion after NO inhibition is consistent with the diuretic/natriuretic capabilities of EETs, as demonstrated by their ability, especially of 5,6-EET, to inhibit Na⁺ transport in the proximal (33) and distal tubules (16) and the collecting duct (35) and to inhibit the hydroosmotic effect of vasopressin (12). Consistent with these effects is the demonstration that EETs activate the Na⁺/H⁺ exchanger in cultured rat glomerular mesangial cells (11), suggesting that EETs may play a role in the regulation of GFR and may thus contribute to the reduction in GFR after NO inhibition.

The selective effect of PPOH and miconazole on the effects of L-NAME on regional renal blood flow, but not blood pressure, in this study suggests that, unlike 20-HETE, the contribution of epoxides to the hemodynamic effects of L-NAME is localized to the kidney and not the systemic circulation. These data do not support our hypothesis of the presence of a prohypertensive epoxide. The discrepancy may reflect the differential in the effects on blood pressure vs. epoxygenase activity of increased endogenous NO production through LPS treatment, producing a 63-mmHg reduction in blood pressure and an 81% inhibition of epoxygenase activity, vs. withdrawal of NO with l-NNA, which produced a 30-mmHg increase in blood pressure and a 20% increase in epoxygenase activity. The observations in this study contrast that obtained in our previous study in which clotrimazole was without effect on L-NAME-induced renal actions of l-NAME (29). This may reflect the low potency and/or poor selectivity of clotrimazole. However, the greater potency and selectivity of epoxygenase inhibitors such as PPOH (4) provide a more definitive tool for truly evaluating a role for epoxides.

Overall, the findings of this study support the interpretation that suppression of NO formation favors functional expression of a CYP epoxygenase product(s) that promotes salt and water excretion and constricts the renal vasculature. These observations are supported by the demonstration that renal intra-arterial infusion of the four regioisomeric epoxides, indeed, produces effects akin to that obtained after inhibition of NO production. Thus 5,6-, 8,9-, 11,12-, and 14,15-EET produced reductions in GFR. The changes in CBF and MBF are generally vasoconstrictive, except for 14,15-EET, which increased MBF at higher doses. In addition, all the epoxides, except 8,9-EET, also produced increases in Na⁺ and water excretion, and this is consistent with their ability to inhibit Na⁺ transport in

Fig. 5. Dose-dependent effects of 11,12-EET (0 (vehicle), 1, 3, and 10 ng/min) on changes in CBF (A) or GFR (B) in rats that received L-NAME (arrow, A) after pretreatment with PPOH (5-mg bolus iv). Renal intra-arterial infusion of 11,12-EET was initiated 5 min before administration of L-NAME (10 mg/kg) in rats (n = 4–5/group) that were pretreated 3 h before administration of 11,12-EET. *P < 0.05 vs. 0 ng/min; *P < 0.05 vs. control.

CYP4A enzymes in the kidney of Dahl rats to 20-hydroxy-11,12- and 14,15-EET (22). Others have described the formation of trihydroxy metabolites of AA from the metabolites of EETs by lipoxygenase and COX enzymes, the latter yielding endoperoxide analogs of EETs (26). Thus it is possible that the renal constrictor effects of EETs in this study derive from their transformation to renal vasoconstrictor products, an observation that is consistent with the conversion of 5,6-EET by platelet-derived COX to vasoconstrictor endoperoxides (9). These observations support the demonstration that EETs activated the Na⁺/H⁺ exchanger in cultured rat glomerular mesangial cells (11), suggesting that EETs may play a role in the regulation of GFR and may thus contribute to the reduction in GFR after NO inhibition.
various segments of the nephron (16, 33, 35). Taken together, these data demonstrate a differential in the renal effects of EETs. The difference in the potencies and actions of the EETs in this study is in agreement with similar findings in the renal circulation. Except for the increase in MBF by 14,15-EET, the reductions in renal blood flow and GFR by the EETs in this study are in agreement with those reported for 5,6- and 8,9-EET (18, 38).

In conclusion, these studies demonstrate that NO inhibits epoxygenase activity, and inhibition of endogenous NOS uncovers a renal vasoconstrictor system operating through the CYP450-dependent epoxygenase metabolism, which contributes to the renal hemodynamic and excretory effects that follow suppression of NOS. It thus appears that 20-HETE and epoxides contribute to the responses following NO withdrawal, but the contribution of epoxides, unlike those of 20-HETE, are limited to the kidney. These findings are applicable only to the acute phase of the response to NOS inhibition in the anesthetized rat; the chronic phase may reflect the operation of a different set of pressor systems (34). Although we do not have supporting hemodynamic and excretory data, the increased epoxygenase activity that was accompanied by increased blood pressure and Na" excretion after a 10-day inhibition of NOS leads us to speculate that EETs may contribute to renal functional changes after long-term inhibition of NO production, probably through a pressure-natriuretic mechanism.

DISCLOSURES

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